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OPERATIONAL NOTE

TEMPORAL AND STORAGE EFFECTS ON ULTRA-LOW VOLUME DROPLETS OF INSECTICIDES COLLECTED ON TEFLON®-COATED SLIDES[1]

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ABSTRACT. Use of microscope slides is the most commonly used method to field-assess the droplet spectrum of ultra-low volume (ULV) sprays. Due to absence of analysis facilities during military deployments, slides must be stored and shipped, and the impact of delays in processing and storage conditions on droplets is unknown. This study was designed to evaluate the effect of storage temperatures and duration on droplets on Teflon®-coated slides. Treatments included BVA-13 mineral oil, Kontrol 30-30 (30% permethrin), and Fyfanon® (96.5% malathion), 2 slide wrapping techniques (proper and improper), and 2 storage temperatures (23 and 45°C), replicated 6 times. The same areas of a slide were measured at different times for 56–58 days using the DropVision® droplet measurement system. Regardless of the wrapping technique, droplets of BVA-13, Fyfanon, and Kontrol 30-30 on slides stored at 45°C reduced significantly after 1, 2, and 1 day, respectively, but droplets on slides stored at 23°C were not significantly affected. The results of this study may assist vector control professionals to accurately interpret the droplet size and help in the effective dispersal of ULV-applied insecticides.

KEY WORDS Droplet durability, evaporation, shrinkage, volume median diameter, reduction

The efficiency of ultra-low volume (ULV) applications depends on airborne insecticidal droplets impacting on flying insects (Sutherland 1983). Droplet size is one of the most important physical factors influencing the efficacy of ULV sprays (Mount et al. 1968, Mount 1970, Sutherland 1983, Curtis and Beidler 1996). Haile et al. (1982) found only slight change in efficiency of ground ULV application of malathion for a droplet size range of 5–25 µm. Tsuda et al. (1987) found housefly knockdown and mortality dependent upon droplet size, with 30 µm as the optimal diameter. Furthermore, they speculated dependence of droplet movement on interaction of inertial force and velocity, the droplet size–dependent factors. Suitable ULV droplet sizes for pesticides are commonly specified on product labels, with emphasis on testing of spray delivery systems for droplet size.

Despite automated droplet measurement systems such as the hot wire probes and lasers gaining popularity, use of glass microscope slides is still the preferred field method (Carroll and Bourg 1979, Dennett et al. 2006). The Department of Defense (DoD) personnel must understand and comply with accurate droplet size and dispersal for effective ULV applications. Military personnel are often deployed to areas where facilities to analyze the slides for droplet size are not available; still the readiness has to be maintained as a DoD principle. Therefore, slides are often shipped for off-site analysis in conditions not ideal for droplet preservation. The potential for errors due to collection, transportation, and storage always exists, but the extent of these errors has not been measured or investigated. Current practices of covering slides with a blank, sealing the edges with a tape, and measuring the droplets within 4 days (Davenport 1976) were not found to be based on scientific evidence. Anderson and Schulte (1971) observed no changes on malathion droplets left undisturbed for 4 days in an air-conditioned room. Moreover, the current practices are time and labor intensive, requiring personnel to facilitate quick reading.

The objective of this study was to evaluate temporal and ambient temperature effects on ULV droplets after deposition on Teflon®-coated slides. The specific objective was to determine potential droplet size reductions over time on Teflon-coated slides under different wrapping and storage conditions.

The experiment was conducted at the Navy Entomology Center of Excellence, Naval Air

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14. ABSTRACT
Use of microscope slides is the most commonly used method to field-assess the droplet spectrum of ultra-low volume (ULV) sprays. Due to absence of analysis facilities during military deployments, slides must be stored and shipped, and the impact of delays in processing and storage conditions on droplets is unknown. This study was designed to evaluate the effect of storage temperatures and duration on droplets on TeflonH-coated slides. Treatments included BVA-13 mineral oil, Kontrol 30-30 (30% permethrin), and FyfanonH (96.5% malathion), 2 slide wrapping techniques (proper and improper) and 2 storage temperatures (23 and 45uC), replicated 6 times. The same areas of a slide were measured at different times for 56?58 days using the DropVisionH droplet measurement system. Regardless of the wrapping technique, droplets of BVA-13, Fyfanon, and Kontrol 30-30 on slides stored at 45uC reduced significantly after 1, 2, and 1 day, respectively, but droplets on slides stored at 23uC were not significantly affected. The results of this study may assist vector control professionals to accurately interpret the droplet size and help in the effective dispersal of ULV-applied insecticides.

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Table 1. Mean droplet characteristics of 3 spray liquids, BVA-13 mineral oil, Fyfanon®, and Kontrol 30-30, at time 0 h and 4 days after spray under various storage and wrapping conditions.

		$Dv_{0.5}$[1] ± SD (μm)					
	Storage	BVA-13		Fyfanon		Kontrol 30-30	
Wrapping	(°C)	0 h	4[2] days	0 h	4 days	0 h	4 days
Proper	23	17.7 ± 0.7a[3]	17.0 ± 0.7a	14.5 ± 0.7a	15.2 ± 1.1a	14.5 ± 0.6a	14.8 ± 0.5a
	45	17.6 ± 1.2a	9.8 ± 2.4b	13.8 ± 0.8a	8.5 ± 1.2b	15.3 ± 1.1a	9.2 ± 2.0b
Improper	23	17.6 ± 0.4a	17.9 ± 0.3a	14.2 ± 0.9a	15.0 ± 1.5a	14.7 ± 0.5a	14.1 ± 0.5a
	45	17.4 ± 0.7a	10.3 ± 1.7b	14.8 ± 0.9a	7.5 ± 0.8b	15.2 ± 0.6a	10.3 ± 1.8b
Open	23	NA[4]	NA	13.6 ± 0.4a	14.0 ± 0.3a	NA	NA

[1] Statistical comparisons for $D_{v0.1}$ and $D_{v0.9}$ were identical to those for $D_{v0.5}$ and thus are not reported here.
[2] Major change in droplets occurred within 4 days and data for 0 h and 4 days are compared.
[3] Means at 0 and 96 h for each combination of factors compared with Student's *t*-test ($\alpha = 0.05$) having different letters are significantly different.
[4] NA, not applicable.

Station, Jacksonville, FL (30°13′N, 81°41′W), from May to August 2011. Spray materials were BVA-13 mineral oil (hereafter BVA; Adapco Inc., Sanford, FL), Fyfanon® ULV (malathion 96.5%; Cheminova Inc., Wayne, NJ), and Masterline® Kontrol 30-30 (permethrin 30.0%; Univar USA Inc., Austin, TX). These materials were sprayed through a truck-mounted Clarke Grizzly® ULV sprayer (Clarke Mosquito Control, Roselle, IL) at a flow rate of 120 ml/min.

Teflon-coated 7.5 × 2.5-cm slides (John W. Hock Company, Gainesville, FL) were waved twice through the spray cloud at 3 m from the nozzle from top to bottom and then from bottom to top in order to collect enough droplets for measurement. A total of 24 slides were waved for each spray material, with 12 slides wrapped both properly and improperly. Proper slide wrapping involved placing a rectangular paper gasket on the sprayed slide, covering it with a clean glass slide, and sealing all the edges with a clear adhesive tape. For improper wrapping of slides, only the longer side of the slide was taped. Slides were grouped into sets of 6, each properly and improperly wrapped for each spray liquid ($n = 36$) and were stored in an oven (model AF30; Quincy Lab Inc., Chicago, IL) at 45°C, whereas the remaining slides ($n = 36$) were stored at 23°C. An additional 6 slides were waved for the Fyfanon application that were not covered at all (open), were placed in the original container with lid closed, and were stored at 23°C. Each slide in the study was considered a replicate. Slides with BVA, Kontrol 30-30, and Fyfanon were sprayed on Mondays of 3 consecutive weeks.

Droplets on the slides were measured at 0 and 4 h and at 1, 2, 3, 4, 7, 14, 21, 28, and 56 days, except the last reading of Fyfanon, which was taken at 58 days. Droplet diameter was measured with DropVision® System (version 2.2; Leading Edge Associates, Waynesville, NC) and the relevant statistics were recorded. The DropVision system parameters used to score an individual droplet were an image size >6 pixels, roundness 1.0, and spread factor from 0.60 to 0.72 as appropriate for each spray material. At 0 h, the droplets were measured from 5–25 spots on the slide or until a minimum of 200 droplets was recorded. The location of each spot on all slides was noted using scales on the DropVision microscope table in order to ensure that all subsequent measurements were taken at the same spots. The droplet size statistics, such as $Dv_{0.5}$, $Dv_{0.1}$, and $Dv_{0.9}$, and total number of droplets were determined. The $Dv_{0.1}$, $Dv_{0.5}$, and $Dv_{0.9}$ are the droplet diameters at which 10%, 50%, and 90% of the spray volume, respectively, consists of droplets smaller than these sizes. When the total number of droplets recorded on a slide was below 50, the subsequent measurements on that slide were terminated.

Analysis of variance was performed with JMP™ statistical software version 5 (JMP, Cary, NC) for the effect of wrapping, temperature, and the delay in reading after collection on droplets. For droplets' longevity under various wrapping and storage temperature combinations, means of the $Dv_{0.1}$, $Dv_{0.5}$, and $Dv_{0.9}$ at any reading time were compared with respective 0-h data, using the *t*-test at 95% level of confidence. The reading time prior to the time when the $Dv_{0.1}$, $Dv_{0.5}$, and $Dv_{0.9}$ differed significantly from the 0-h measurement was defined as the stability period for that size.

As some of the slides only lasted for 4 days and most of the change in droplet size for other slides stored at 45°C was observed within 4 days, only the comparison of various sizes under different conditions between time 0 h and 4 days was done (Table 1). For all spray liquids, the reduction in $Dv_{0.1}$, $Dv_{0.5}$, and $Dv_{0.9}$ over time for slides stored at 45°C was much more abrupt compared to that of slides stored at 23°C. By day 21, the droplets on all slides from BVA spray stored at 45°C dropped below 50 and further measurements on these slides were curtailed. The change in $Dv_{0.1}$, $Dv_{0.5}$, and $Dv_{0.9}$ for BVA slides stored at 23°C with time was not significant for 21 days. Furthermore, the difference in droplet parameters

Table 2. Days elapsed before $D_{v0.5}$[1] significantly changed from 0 h when compared with Student's t-test ($\alpha = 0.05$) for 3 spray liquids (BVA-13 mineral oil, Fyfanon®, and Kontrol 30-30), wrappings, and storage conditions.

Wrapping	Storage (°C)	Spray liquid BVA-13	Fyfanon	Kontrol 30-30
Proper	23	21	>58	>56
	45	1	1	1
Improper	23	21	>58	>56
	45	1	2	1
Open	23	NA[2]	58	NA

[1] Statistical comparisons for $D_{v0.1}$ and $D_{v0.9}$ were identical to those for $D_{v0.5}$ and thus are not reported here.
[2] NA, not applicable.

of properly and improperly wrapped BVA slides under both storage conditions was not significant for >4 days (Table 1). For BVA slides, comparison of $Dv_{0.1}$, $Dv_{0.5}$, and $Dv_{0.9}$ at any time with the diameter at 0 h (original size) indicated that these parameters significantly decreased from original size by day 1 for slides at 45°C and by 21 days for slides stored at 23°C (Table 2).

For Fyfanon, the number of droplets on slides stored at 45°C decreased to <50 by day 4 and further measurements were stopped. Overall, the $Dv_{0.1}$, $Dv_{0.5}$, and $Dv_{0.9}$ for slides stored at 23°C were not significantly affected by time (Table 1). The difference in droplet parameters of properly and improperly wrapped slides under both storage conditions throughout the measurement times was not significant. The droplets of open slides became significantly smaller than those of wrapped slides at 23°C after 28 days. The $Dv_{0.1}$, $Dv_{0.5}$, and $Dv_{0.9}$ for slides stored at 45°C significantly decreased from the diameter at 0 h in 1–2 days (Table 2). Among slides stored at 23°C, these parameters did not significantly change over the study period of 58 days for properly and improperly wrapped slides, whereas it changed significantly in 58 days for open slides (Table 2).

The number of droplets on slides from Kontrol 30-30 stored at 45°C decreased to <50 by day 21 and further measurements discontinued. There were some significant yet sporadic differences in droplet parameters from properly and improperly wrapped slides at 45°C, possibly due to

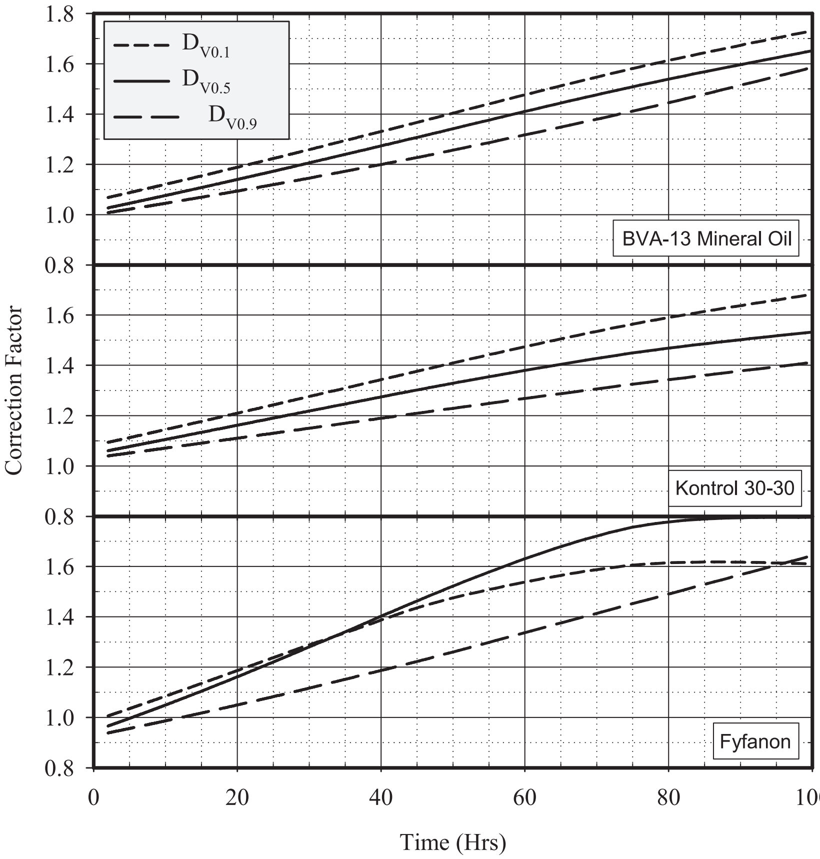

Fig. 1. Correction factor for $Dv_{0.1}$, $Dv_{0.5}$, and $Dv_{0.9}$ of slides for 3 spray liquids stored at 45°C for different hours of delay in reading.

measurement errors. These differences for slides at 23°C were not significant throughout the study period. The $Dv_{0.1}$, $Dv_{0.5}$, and $Dv_{0.9}$ for slides stored at 23°C were not significantly affected by time. Comparison of $Dv_{0.1}$, $Dv_{0.5}$, and $Dv_{0.9}$ at any time to the diameter at 0 h indicated that these values significantly decreased in 1 day for slides at 45°C and did not significantly change during the study period of 56 days for properly and improperly wrapped slides stored at 23°C (Table 2).

Wrapping the slides did not have a significant impact on droplet size parameters throughout the delay times of 56–58 days in this study except for open slides stored at 23°C, which showed a reduction in size after 28 days. The temperature had a strong effect on droplet size, which varied with spray liquids. Hence, it is concluded that for reading locally or for short-distance supervised transportation, covering and wrapping the slides is not required. After collection, slides should be stored in their box, the box closed to avoid droplet distortion and evaporation, and then stored at room temperature. These slides can be read within 21 days without significant effect on droplet size. For long-distance unsupervised transportation, we suggest covering and moving slides to a cooler place. It is also advised to store slides at room temperature and to ship in insulated containers.

This study has shown that droplet size reduced within a few hours at 45°C and temperature around this can be encountered during droplet characterization at many field sites. Upon exposure of slides to such conditions, the chances of an error increase and the need to correct these errors was recognized. The correction factors for 3 spray liquids using the droplet size data for slides stored at 45°C are presented in Fig. 1. If the exposure to heat lasts for >1 h, record elapsed time and adjust measured diameter by multiplying the right correction factor from Fig. 1. It should be remembered that these correction factors apply only to the chemicals and conditions tested in this study.

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